Dermal absorption of pesticide residues

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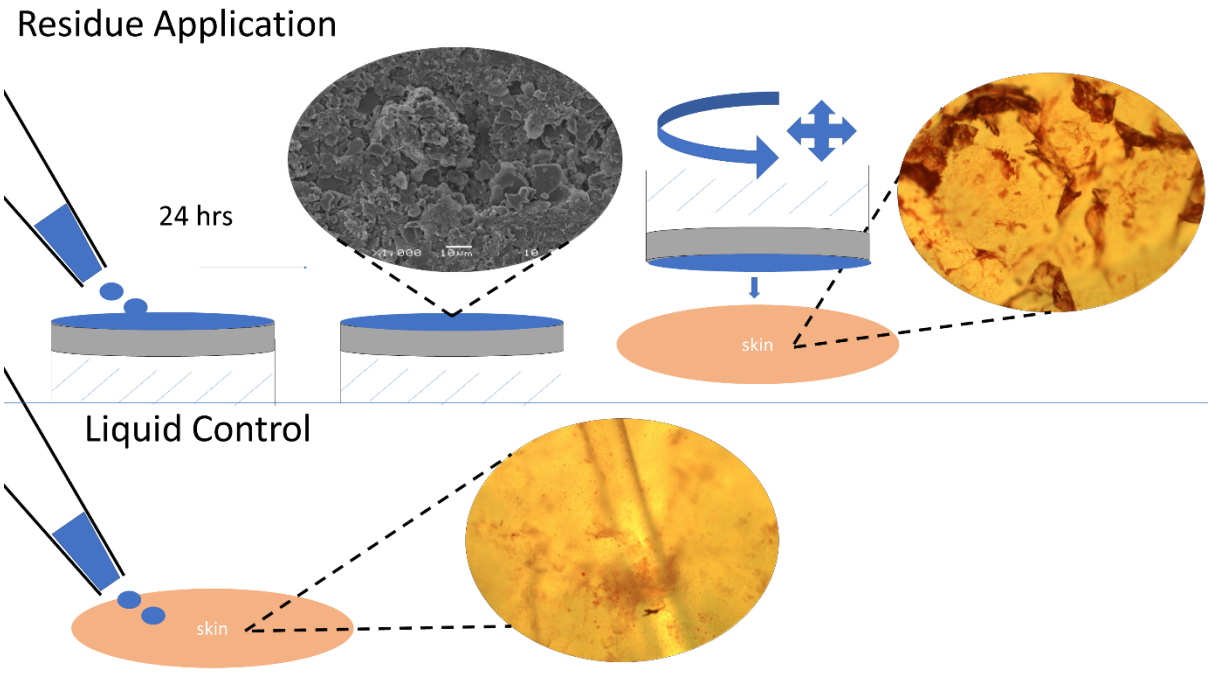
ToC graphic



Abstract

Current guidance for dermal exposure assessment of plant protection products typically uses *in vitro* skin penetration data for the active ingredient when applied as both the concentrated product and relevant spray dilutions thereof. However, typical re-entry scenarios involve potential skin exposure to a 'dried residue' of the spray dilution, from which the absorption of a pesticide may be quite different. The research reported in this paper has shown: [1] The method to assess the transfer of dried pesticide residues from a surface to the skin is reproducible for four active ingredients of diverse physicochemical properties, after their application in commercially relevant formulations. [2] Skin absorption of all four pesticides examined was significantly less from a dried residue than from a spray dilution; the difference, in general, was of the order of a factor of 2. [3] Decontamination experiments with one of the active ingredients tested (trinexapac-ethyl) showed that, post-exposure to a spray dilution, skin surface cleaning must be performed within 1 hour to significantly reduce potential systemic exposure (relative to continual contact for 24 hours); in contrast, after contact with a dried residue, the sooner decontamination was performed, the greater the decrease in exposure achieved, even when the time of contact was as long as 8 hours.

Keywords: dermal absorption, pesticide, risk assessment, re-entry, residue

Introduction

When pesticides are used in practice, the application is usually in the form of a 'spray dilution' in which the concentrated formulation has been mixed with water. After application, the diluted formulation eventually leaves a 'dried residue' on surfaces such as leaves or fruit. For each pesticide product, a series of risk assessments must be carried out before use. These calculations encompass various scenarios and consider the product's effect on the ecosystem and on a number of specific organisms, including humans. For example, an operator could potentially be exposed during mixing and loading of the product into a spray tank and, during application there is a further possibility of exposure, not only to the operator but also to bystanders and nearby residents; in addition, anyone entering the treated area post-application may be at risk of exposure to the recently applied product.

A re-entry worker, an individual who enters a field to carry out a task such as crop inspection or harvest, may enter a treated area soon after pesticide application and risk exposure, therefore, to the dried residue remaining on leaves, fruit, etc. Exposure occurs most typically via dermal (the most important[1]) and inhalation routes, with secondary exposure also possible via hand-to-mouth transfer. The potential dermal exposure (PDE) can be estimated for the purpose of a risk assessment and depends upon: (a) how much pesticide is present on the contaminated surface, the so-called dislodgeable foliar residue (DFR), dictated by factors such as application rate of the active ingredient (AI), and the pesticide formulation; (b) how much of the DFR is subsequently transferred to the skin, as characterised by a transfer coefficient (TC), dictated by the degree of contact between the worker and the contaminated surface; and (c) the duration of exposure. Once the PDE has been determined, the quantity of pesticide which will be absorbed[*] through the skin and eventually become systemically available can be estimated.

[*] Throughout this article the term 'absorption' is used to describe a combination of both uptake and penetration, where 'uptake' refers to active ingredient (AI) which partitions into the skin tissue, and 'penetration' refers to AI that passes through the tissue into the receptor solution.

The skin's primary function is to act as a barrier both to the loss of water and to the absorption of xenobiotics. The outermost layer, the non-viable stratum corneum (SC), is typically the rate-limiting barrier to absorption, meaning that *ex-vivo* skin has a competent SC[2] and can be used *in vitro* as a surrogate for the *in vivo* situation[3]. Consequently, at present, to determine the skin absorption of a pesticide, *in vitro* diffusion cell experiments on the concentrated product, and on representative spray dilutions, are performed. The highest fraction of the 'dose' absorbed from the formulations is then used for risk assessment, and this value is multiplied by the PDE to yield an estimate of systemic exposure. The use of human skin is a requirement for these studies[1] as the purpose is to obtain absolute absorption values; it follows that the best estimate of the *in vivo* absorption will be obtained using human, as opposed to an animal model's skin.

However, as articulated above, in a re-entry scenario, a worker does not come into contact with the concentrate or a spray dilution of the pesticide product; rather, skin contact occurs with the dried residue of the spray dilution. In previous experiments, a significant difference was observed in the dermal absorption of various pesticides from liquid and residue forms[4]; in some cases, the chemical was absorbed more from the residue but, in others, the uptake was less. The *in vitro* method used to measure skin uptake from the residue involved pesticide application to a disc made from an artificial material, the coated surface of which was subsequently pressed against the skin for 8 hours (representing a typical working day). An obvious limitation of this approach is that the skin is occluded by the disc throughout the exposure, and increased hydration has been shown to amplify dermal absorption[5-7]. This effect may be exacerbated for a dried residue, as surface moisture resulting from occlusion effectively becomes the 'vehicle'. Another shortcoming of the protocol used was that the active ingredient was deposited on the artificial surface from a simple solvent rather than from a commercially relevant formulation. Lastly, the lowest dose used was at the upper end of a realistic worker exposure. As many studies have shown percentage absorption often decreases with increasing skin loading[8-11], the use of doses higher than those to which a worker may be exposed may provide unrealistic absorption values.

To overcome these limitations, an improved experimental methodology has been reported for the measurement of pesticide dermal absorption from a dried residue *in vitro*[12]. It was shown that the skin uptake and absorption of trinexapac-ethyl from the spray dilution of a naphtha-based emulsifiable concentrate (EC) formulation was significantly higher than that from its dried residue. This initial proof-of-concept, however, involved one pesticide, at a single dermal loading, and applied from one formulation. The research described in the present study aims at a broader scope so that firmer conclusions may be drawn about the effects of formulation and dermal loading on the skin penetration of pesticides from dried residues.

This refined approach has therefore been used to determine and compare the dermal absorption of liquid and residue forms of four pesticides from various formulations and dose levels. Furthermore, for one of the chemicals, the effect of decontamination after different exposure periods has been investigated to better simulate, for example, hand-washing events prior to rest or lunch breaks in a worker's typical 8-hour day. Clearly, hand-washing can potentially remove a significant fraction of a dried residue and therefore reduce the overall systemic exposure[13]. Although it has been found that hand-washing does not completely decontaminate the skin[14,15], perhaps because mobilisation of material trapped in skin crevices and/or appendages is difficult and less than 100% efficient, the extent to which similar behaviour occurs with dried residues is presently unknown. It could be speculated that decontamination of a residue may be more efficient as it would not 'flow' into skin crevices.

Overall, therefore, the aim of this study was to compare absorption of pesticides from residue and liquid applications using a porcine skin model and to further test the methodology for residue application. However, absolute absorption values suitable for risk assessment are not reported here as their evaluation would require extensive and systematic experiments using human skin from multiple donors.

Methods

Materials

Four active ingredients, spanning a range of physicochemical properties, were considered: trinexapac-ethyl (TXP), clodinafop-propargyl (CLF), difenoconazole (DFZ) and propiconazole (PPZ) (Table 1). The pesticide formulations examined (Syngenta, Jealott's Hill, UK), were two emulsifiable concentrate formulations (EC-A and EC-B) which were diluted 100-fold in water to provide nominal spray dilution concentrations of the active ingredient of 1 mg/mL, and a wettable powder (WP) containing the pesticide 15% w/w. To prepare a spray dilution of the WP at a concentration of 1 mg/mL, 667 mg were made into a paste, passed through a sieve (Endecotts, London, UK) of aperture 125 µm, and then made up to 100 mL in water. As the resulting formulation was a suspension, thorough vortex mixing was performed before use.*Table 1: Pesticides selected for investigation and their relevant physicochemical properties.*

Compound	Molecular weight	Melting point (°C)	log P[a]	Aqueous solubility (mg/cm^3)	Predicted J_{max}[b] ($\mu g/cm^2/h$)[16, 17]
Trinexapac-ethyl (TXP)	252	36.3	-0.29	10.2	0.366
Clodinafop-propargyl (CLF)	350	59.5	3.9	0.004	0.032
Difenoconazole (DFZ)	406	82.5	4.36	0.015	0.117
Propiconazole (PPZ)	342	-23	3.72	0.15	1.02

[a]P = octanol-water partition coefficient. [b]Maximum skin penetration flux following an infinite dose of a saturated aqueous solution.

Measurement of dermal absorption

In vitro skin absorption experiments were performed using static Franz diffusion cells with a receptor volume of 7.4 mL and area of 2 cm^2. Dorsal porcine skin was obtained from a local abattoir. Within each subset of experiments, skin from a single donor was used for all measurements. Skin was dermatomed (~750 µm, Zimmer, USA), frozen within 24 hours of slaughter and thawed before use. Porcine skin was chosen as it is considered to be the closest surrogate to human skin[18-20]. Using skin from the same animal for each series of experiments reduced variability in the results and facilitated comparison between different conditions. The method used was based upon OECD[21] and EFSA[22, 23] guidance documents for *in vitro* diffusion cell studies required in the regulatory approval process. The only significant deviation was that, instead of a water jacket system to control skin temperature, diffusion cells were incubated at 32 ± 1^0C, and at a relative humidity of 40 ± 5% in a controlled environment cabinet (and, in particular, the humidity, which is dependent on the time of day and fluctuates seasonally). Tighter control of the environmental conditions at the surface of the skin was considered important for this study because of the potentially rate-limiting nature of the dissolution kinetics of the residue.

Effect of active ingredient

A spray dilution of the EC-A formulation of each of the four AIs at a representative concentration, was either (i) applied directly to the skin as a 'liquid', or (ii) dried to form a residue and subsequently applied to the skin.

For the liquid control, a volume of 25-30 µL* was typically applied for a period of 8 hours (a 'working' day), after which the skin surface was cleaned. The wash procedure involved applying 100 µL of a 0.1% w/v soap solution to the skin and cleaning with two cotton swabs, from which the AI was subsequently extracted and quantified. The receptor solution (which consisted of phosphate-buffered saline (PBS) pH 7.4, with or without 6% Volpo™ (Sigma Aldrich Co., Gillingham, UK)) was sampled each hour from 2 to 8 hours for TXP and then at 24 hours. For the other three AIs, receptor solution samples were taken at 8 and 24 hours only. After the final receptor solution sampling at 24 hours, the skin was removed from the diffusion cell and the stratum corneum was removed by adhesive tape-stripping (Scotch Book Tape, 3M, Germany) as previously described[4]. According to the EFSA guidelines[22], AI in the first two tape strips is considered to be non-absorbed material that would be lost *via* desquamation, while that in the subsequently removed 13 tape strips is assumed to be absorbed, as is the pesticide recovered from the remaining skin tissue post-stripping. The methods used to ensure efficient extraction of the AIs from the stratum corneum tape-strips and from the remaining skin post-stripping are described in the Supplementary Information, as are the HPLC analytical protocols for each AI. 'Total absorption' of the AI was determined as the amount of pesticide in stratum corneum tape-strips 3-15, plus in the skin remaining post-stripping, plus in the receptor solution at 24 hours (taking into account pesticide removed during earlier samplings). AI in the first two stratum corneum tape-strips was extracted and quantified but not included in the total absorption reported.

To measure AI absorption from a 'dried' residue, exactly 40 µL of the liquid spray dilution was applied to a stainless-steel disc of 12 mm diameter (SPM specimen discs, TAAB Laboratories Equipment Ltd., Aldermaston, UK) and allowed to dry for 24 hours. Additional discs were prepared in exactly the same manner, three of which from each batch were extracted for the AI after the drying period; this enabled

* The exact volume used was informed by the average amount of AI residue transferred to the skin from the stainless-steel disc to which 40 µg had been applied (see following text) in preliminary experiments. The actual volumes in the control experiments were 25 µL for TXP and PPZ, and 30 µL for DFZ and CLF.

confirmation that the correct amount of AI had been applied to each disc and that no evaporation of AI had occurred.

This residue was then applied to the skin with a standardised transfer procedure[12]. AI remaining on the disc post-transfer was then extracted and quantified allowing the mass of AI transferred to each piece of skin to be determined by difference. After application of the residue to the skin, the *in vitro* diffusion cell experimental protocol was identical to that for the liquid application explained above. The exact movements of the transfer procedure are not necessarily of great importance, so long as the movement of the disc upon the skin is sufficiently varied to dislodge a sufficient proportion of the residue and spread this across the entire skin surface. This movement should be consistent each time to reduce variability in the amount applied.

Effect of formulation

Two pesticides were considered: (a) clodinafop-propargyl (CLF) and (b) trinexapac-ethyl (TXP). Identical procedures to those already described were used except that each compound was applied from EC-A, EC-B and WP formulations and the skin used was from a different pig.

Effect of dose

In addition, both TXP and CLF were investigated at various 'dose' levels: (a) 30, 70 and 120 µL of TXP EC-A spray dilution were applied, while the corresponding residue discs were loaded with 40, 100 and 180 µL of the same spray dilution to match the dose levels (i.e., applying the same mass of active ingredient per cm^2 of skin); (b) 30, 70, 120 and 160 µL of CLF EC-A spray dilution were applied and residue discs were loaded with 40, 100, 180 and 250 µL.

Effect of exposure duration

For TXP, additional skin uptake experiments were performed to assess the impact of skin washing after different exposure periods. Identical procedures to those already described were used with three modifications: (i) the skin surface was cleaned after separate exposure durations of 0.5, 1, 2, 4, 8 and 24 hr; (ii) the receptor solution was phosphate-buffered saline alone; and (iii) the skin used was from another pig.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, California, USA). Students T-test, and 1-way and 2-way ANOVAs were carried out as appropriate (see below).

Results & Discussion

Effect of active ingredient

The 24-hour skin absorption of the four AIs considered is summarised in Figure 1. More detailed skin absorption data are shown in Table 2. Application of residue from the transfer procedure was consistent and well-matched to the liquid control applications. Residue applications of TXP and PPZ were 22.5 ± 4.3 and 24.4 ± 1.3 µg respectively, as compared to the 25 µg applied from their spray dilution controls. Applications of DFZ and CLF residues were respectively 29.0 ± 3.4 and 30.0 ± 2.6 µg in comparison to the 30 µg applied from the spray dilutions.

Given the close overlap between the amounts of AIs applied, expression of the data in terms of the % of the applied 'dose' is a valid comparison between the chemicals, and between absorption from liquid and residue phases. For all four AIs, the total % 'dose' absorbed was significantly higher ($p < 0.05$) from the liquid than from the residue. Across the different AIs, for both liquid and residue applications, there was about a 2- to 3-fold range in the absolute skin absorption values.

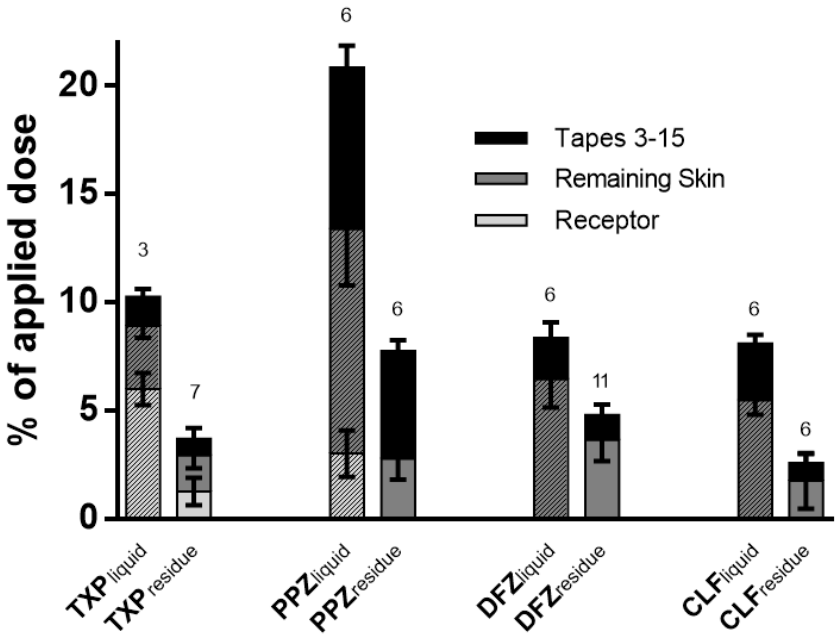


Figure 1: Skin uptake into and across pig skin in vitro at 24 hours after liquid (hatched bars) and residue (plain bars) application (8-hour exposure) *of the pesticides considered (mean ± SD; the number of replicates of each experiment is indicated above each bar on the figure).*

From the liquid spray dilution application, TXP penetration through the skin to the receptor phase was detectable from the first measurement at 2 hours and increased progressively over the duration of the experiment (Figure 2(a)). The derived rate of penetration, however, indicated a clear maximum at around 4 hours (Figure 2(b)). Given that only ~10% of the applied TXP was absorbed over the entire 24 hours of measurement, the peak in the absorption rate cannot be attributed to depletion of the dose applied. Rather, it is more likely that the downturn in the rate of penetration is the result of the liquid spray dilution drying out and leaving a solid residue after a certain time from which absorption is much slower; that is, the pesticide now needs to re-dissolve in the limited surface moisture available before it can diffuse into the skin. The results from the residue application support this interpretation. In this case, TXP was only detected in the receptor at 24 hours, indicative of a much longer lag-time (and reflecting the slow dissolution step referred to above). This is consistent with a previous study[12] involving a similar protocol. This study involved an additional set of experiments where the skin was not washed until 24 hours post-application; an early peak rate of absorption was again seen for the liquid application but, over the 8-24 hour period, the fluxes of TXP from the liquid and from the residue were essentially the same (0.08 and 0.09 µg/hr, respectively).

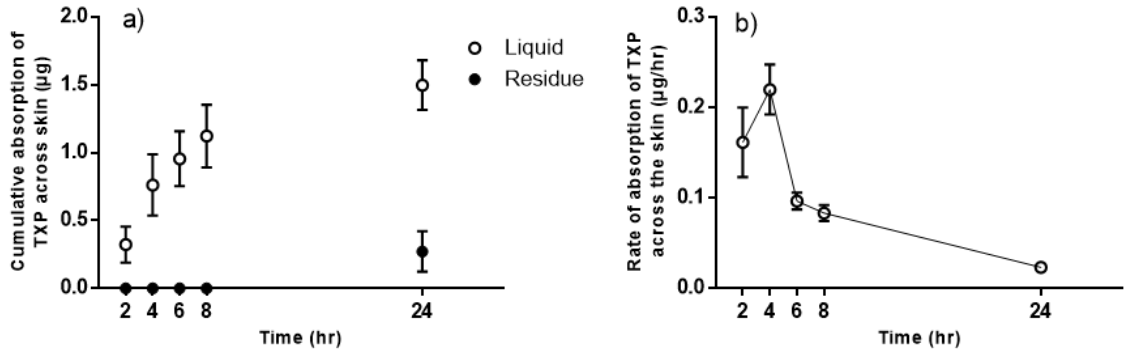


Figure 2: (a) Cumulative absorption of TXP across the skin in vitro (8-hour exposure) (mean ± SD) following liquid spray dilution (n = 3) and residue (n = 7) applications as a function of time. (b) Rate of absorption of TXP across the skin in vitro following application of a liquid spray dilution as a function of time (mean ± SD; n = 3).

In terms of the overall skin disposition of TXP, the total absorption (expressed either as an absolute quantity or as a % of the dose applied) was 2.8 times larger from the liquid spray dilution than from the residue; this difference was significant at $p < 0.01$. Differences in amounts recovered from the stratum corneum tape-strips and the rest of the skin were somewhat smaller (closer to 2-fold) but were again statistically significant.

As indicated in Table 2, PPZ was the most efficiently taken up of the four pesticides from both liquid and residue applications. PPZ and was absorbed 2.4 times more when applied in a liquid (20.8% and 8.5%, respectively, in terms of % dose applied, a significant difference at $p < 0.01$). Penetration of the chemical to the receptor phase, however, was measurable for the liquid spray dilution application only at 24 hours; nothing was detectable at 8 hours. For the residue, PPZ in the receptor did not reach the LOQ at either 8 or 24 hours. Uptake of PPZ into the skin – both in terms of the amounts in stratum corneum tape-strips 3-15 and in the remaining skin – was significantly higher ($p < 0.01$) for the liquid application. This was slightly surprising as the melting point of this chemical is -23°C (indicating that it is a liquid at ambient temperature) and therefore, when left as a residue, a lesser resistance to dissolution might have been expected than for a compound that is a solid at this temperature.

The results for DFZ and CLF were relatively similar. For both chemicals, skin uptake was significantly higher ($p < 0.01$) from the liquid spray application than from the residue by factors of approximately 1.8 (DFZ) and 3.1 (CLF). Neither pesticide was detectable in the receptor solution after 24h, regardless of application method but this could not be attributed to limited solubility; in fact, the solubilities of DFZ and CLF in the receptor phase were 1.5 and 0.5 mg/mL, respectively). It should be noted, however, that the limits of quantitation (LOQs) for DFZ and CLF were 0.025 and 0.035 µg/mL, respectively; this means that, with a receptor volume of 7.5 mL, there could be as much as 0.26 µg (i.e., ~1% of the applied 'dose') to be present in samples reported as below the LOQ. As the total percentage absorbed

was as low as 2% in some replicates, this is potentially of significance. For trinexapac-ethyl and propiconazole, the LOQs are 0.03 and 0.07 µg/mL, respectively; again, therefore, care should be taken when assessing data from the residue applications, a number of which were below the LOQ.

Table 2: Skin absorption of the four active ingredients (AIs) considered (8-hour exposure) (mean ± SD; n = 3-6 for the liquid application, n = 6-11 for the residue).

	Trinexapac-ethyl		**Propiconazole**		**Difenoconzole**		**Clodinafop Propargyl**	
Application	**Liquid**	**Residue**	**Liquid**	**Residue**	**Liquid**	**Residue**	**Liquid**	**Residue**
AI applied (µg)	25	22.45 ± 4.25	25	24.44 ± 1.28	30	29.01 ± 3.44	30	29.97 ± 2.64
AI recovered in swabs (µg)	11.12 ± 1.63	11.06 ± 2.38	12.59 ± 3.30	19.95 ± 1.53[§]	24.31 ± 1.88	22.45 ± 3.32	21.09 ± 2.56	23.74 ± 4.81
AI disposition								
SC tape-strips 1-2 (µg)	1.20 ± 0.28	0.53 ± 0.24[*]	2.83 ± 1.22	1.87 ± 0.40	1.55 ± 0.59	1.16 ± 0.33	2.24 ± 0.47	1.25 ± 0.52[*]
SC tape-strips 3-15 (µg)	0.33 ± 0.09	0.17 ± 0.12	1.86 ± 0.25	1.33 ± 0.16[*]	0.57 ± 0.21	0.34 ± 0.15[†]	0.79 ± 0.12	0.25 ± 0.14[*]
SC tape-strips 1-15 (µg)	1.53 ± 0.37	0.70 ± 0.33[*]	4.69 ± 1.31	3.20 ± 0.47[†]	2.12 ± 0.64	1.49 ± 0.45[†]	3.03 ± 0.42	1.50 ± 0.65[*]
Remaining skin (µg)	0.73 ± 0.14	0.38 ± 0.16[*]	2.58 ± 0.65	0.75 ± 0.26[*]	1.93 ± 0.39	1.05 ± 0.28[*]	1.64 ± 0.19	0.54 ± 0.41[*]
Receptor phase at 2 hr (µg)	0.32 ± 0.13	< LOQ[@]	NA	NA	NA	NA	NA	NA
Receptor phase at 4 hr (µg)	0.76 ± 0.22	< LOQ	NA	NA	NA	NA	NA	NA

Receptor phase at 6 hr (µg)	0.96 ± 0.20	< LOQ	NA	NA	NA	NA	NA	NA
Receptor phase at 8 hr (µg)	1.12 ± 0.23	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Receptor phase at 24 hr (µg)	1.50 ± 0.18	0.27 ± 0.15*	0.75 ± 0.27	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
Total AI uptake/absorption (µg)#	2.56 ± 0.18	0.82 ± 0.34*	5.19 ± 0.99	2.08 ± 0.37*	2.50 ± 0.52	1.38 ± 0.35*	2.43 ± 0.30	0.79 ± 0.47*
% uptake/absorption of AI applied	10.3 ± 0.4	3.7 ± 1.4*	20.8 ± 4.0	8.5 ± 1.4*	8.3 ± 1.7	4.8 ± 1.1*	8.1 ± 1.0	2.6 ± 1.4*
Total Recovery (%)	66.5 ± 7.4	75.8 ± 8.5	85.2 ± 15.4	100.8 ± 2.0	96.9 ± 5.1	87.8 ± 4.4	88.7 ± 9.8	91.0 ± 9.8

§Significantly greater ($p < 0.01$) than the liquid application value (Student's unpaired t-test).

*Significantly smaller ($p < 0.01$) than the liquid application value (Student's unpaired t-test).

†Significantly smaller ($p < 0.05$) than the liquid application value (Student's unpaired t-test).

@< LOQ = below the limit of quantitation

#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

Effect of formulation

CLF and TXP were applied to the skin from three commercial formulations, each as a liquid spray dilution and as a residue. For comparison (without formulation excipients), TXP was also applied from an aqueous solution and from a dried residue thereof; CLF was additionally applied as a residue remaining after evaporation of an acetone solution of the chemical.

The CLF residue transferred to the skin surface depended on the formulation used; the average amounts applied were: 22.6 (± 1.8) µg for EC-A, 26.2 (± 1.3) µg for EC-B, 35.2 (± 1.96) µg for WP, and 17.9 (± 3.3) µg for the AI deposited from acetone. These quantities were used when calculating percentages of the applied dose absorbed. No CLF was detected in the diffusion cell receptor solution from any application. The results are summarised in Figure 3 and (in tabular form) in the Supplementary Information.

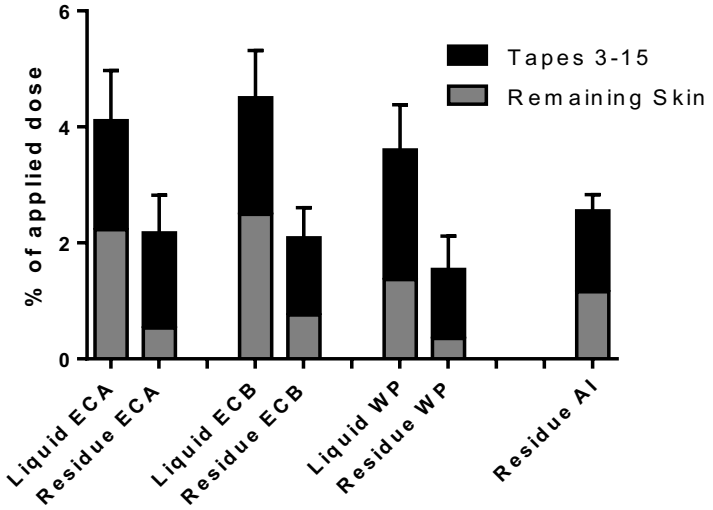


Figure 3: Skin uptake of CLF at 24 hours after liquid and residue applications (8-hour exposure) from different formulations (mean ± SD; n = 4-8).

Formulation type had no significant impact on CLF uptake for the liquid application.. However, when the estimated amount applied for each formulation was taken into account, % uptake/absorption from residues was significantly different between formulations (1-way ANOVA, $p < 0.01$). Formulation

also influenced the amount of CLF recovered from the stratum corneum on tapes 1 and 2; notably, if these quantities were included in the % uptake/absorption, no significant difference was observed between formulations. This information is potentially important because the amount of pesticide recovered from the first two tape-strips was greater than that recovered from the remaining tapes and skin tissue combined. Hence, when one discards this chemical from the % amount taken up and absorbed, the effect is substantial and is quite different than that found for TXP, which has been demonstrated to be absorbed across the skin much more quickly than CLF. In previous studies (unpublished), it was observed that the AI used did not affect either the total mass of stratum corneum removed, or that removed by the first two tapes when applied in EC-A. However, the extent to which other types of formulation (e.g., drier products such as the WP) could cause different amounts of SC to be removed is a subject for further investigation.

Transfer of TXP residue was not significantly different between the three commercial formulations: 27.1 (± 1.5) µg for EC-A, 29.1 (± 3.1) µg for EC-B, 27.3 (± 5.7) µg for WP. However, the residue from an aqueous solution was transferred considerably less (11.3 (± 0.96) µg). The fact that transfer of both pesticides was more efficient from the commercial formulations than from simple solutions of the chemical (water for TXP, acetone for CLF), may reflect the role of excipients (e.g., surfactants) in facilitating solubilisation of the active ingredients.

A 2-way ANOVA revealed that skin absorption of TXP was significantly different between formulations and application type ($p < 0.001$). There was also an interaction between these two variables ($p < 0.01$). TXP was absorbed significantly less (relative to the liquid spray dilutions) when applied as a residue from EC-A and EC-B, but not from WP (Figure 4). Penetration of TXP from the spray dilutions was significantly different between formulations. On the other hand, the amounts of TXP recovered from the stratum corneum and the remaining skin tissue were similar between the formulations.

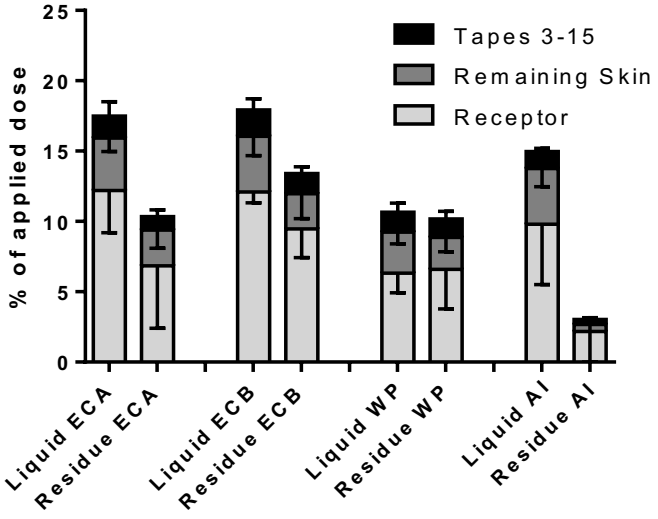


Figure 4: Skin absorption of TXP at 24 hours after liquid and residue applications (8-hour exposure) from different formulations (mean ± SD, n = 3-9).

Effect of dose

In a further set of experiments, the skin uptake and penetration of CLF and TXP from EC-A were determined as a function of the applied quantities of the pesticide following both liquid spray dilution and residue applications.

The total skin absorption of CLF increased linearly with increasing quantity of the chemical applied for both the liquid and residue applications (Figure 5(a); r^2 = 0.97 and 0.94, respectively). In terms of the % 'dose' absorbed, a 2-way ANOVA revealed that, while more CLF was absorbed from the liquid, there was no significant effect of dose loading on either method of application (Figure 5(b)). There was, however, a significant interaction between the two variables (i.e., the nature of the application and the skin 'loading'). Interestingly, when the quantities of CLF recovered in the first two stratum corneum tape-strips were included in the total skin absorption calculation, the 2-way ANOVA found that the chemical loading did have a significant effect of the % 'dose' absorbed, especially for the liquid application (Figure 5(c)). The complete dataset is provided in the Supplementary Information.

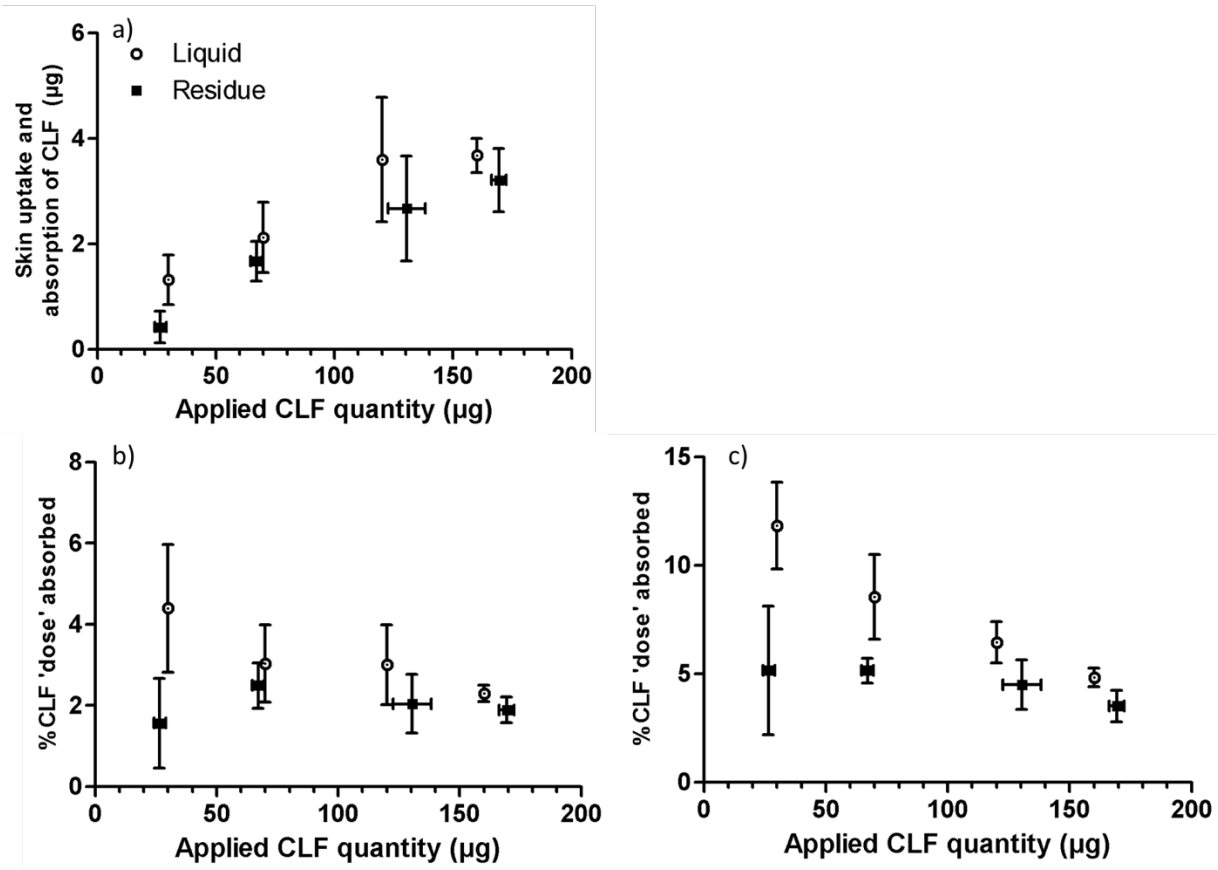


Figure 5: CLF uptake into the skin (mean ± SD; n = 6 - 13*) at 24 hours following 8-hour liquid and residue applications as a function of chemical loading ('applied dose') expressed (a) as the absolute quantity (mass), (b) as the % of the applied 'dose', and (c) as in (b) but including the pesticide recovered in the first two stratum corneum tape-strips (TS 1-2).*

The total skin absorption of TXP also increased linearly with increasing quantity of the chemical applied for both the liquid and residue applications (Figure 6(a); r^2 = 0.94 and 0.82, respectively). In terms of the % 'dose' absorbed, there was no significant effect of dose loading for either method of application (Figure 6(b)).

It is worth pointing out that the lowest liquid application of 30 µL (i.e., 15 µL/cm^2 given the area of skin used) is approximately the minimum volume needed to just cover the skin surface. This slightly higher 'dose' than the 10 µL/cm^2 recommended by EFSA[22] was used due to concerns about quantification of the compounds given their limited ability to be absorbed across the skin. Larger

loadings, or volumes, do not therefore increase the area of contact of the formulation with the skin, but do provide a greater reservoir of the penetrant. The increased loadings also deposit more of the solvent from the liquid spray dilutions and prolong, as a result (and, as was discussed before) the time before the 'metamorphosis' of the deposited material is complete. Again, as previously explained, this behaviour is much less, if at all, apparent with the residues for which no solvent remains. Regardless of the loading, the skin absorption of pesticide over 24 hours never exceeded 30% of the applied 'dose'; that is, an insufficient depletion to account for the slowing permeation of the chemicals as the experiment proceeded.

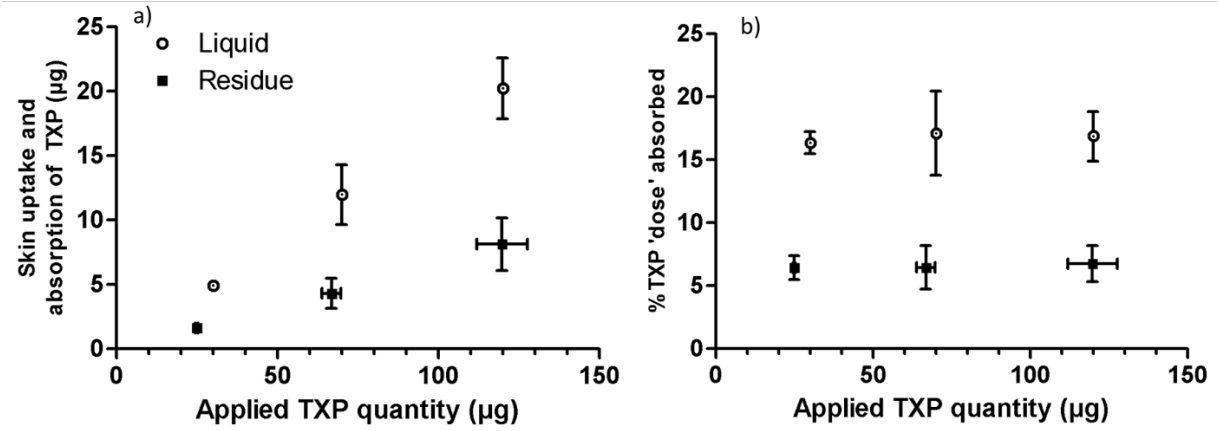


Figure 6: TXP absorption into the skin (mean ± SD; n = 4) at 24 hours following 8-hour liquid and residue applications as a function of chemical loading ('applied dose') expressed (a) as the absolute quantity (mass), and (b) as the % of the applied 'dose'.

Effect of exposure duration

TXP absorption from liquid and residue applications, when the skin was cleaned after different exposure periods, is summarised in Figure 7. Tabulated results are in the Supplementary Information.

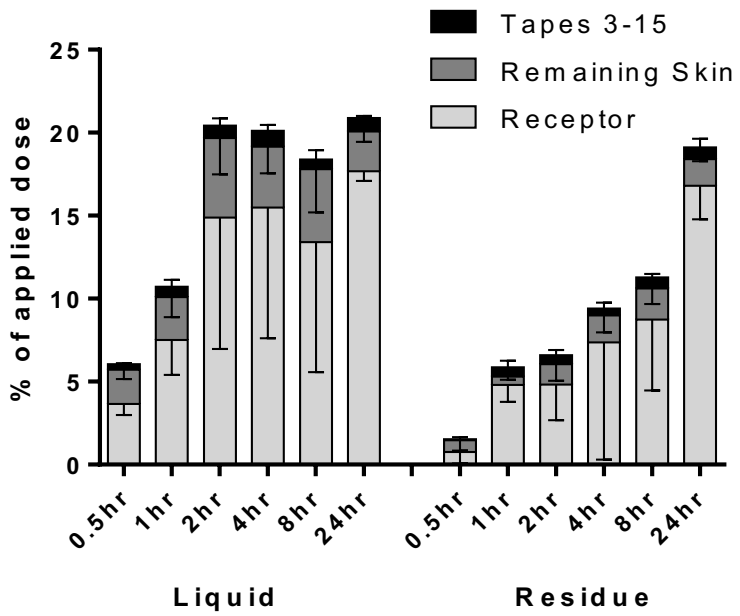


Figure 7: Stacked bar chart showing skin absorption of TXP after 24 hours when the skin surface had been decontaminated after exposure periods of 0.5, 1, 2 ,4, 8 and 24 hours (mean ± SD; n = 3-7)

A 2-way analysis of variance (ANOVA) of these results shows that the time of decontamination had a significant impact on the % of the applied amount of TXP absorbed ($p < 0.001$), as did the application method (liquid versus residue) ($p < 0.0001$). The interaction between the two variables, however, was not significant.

The total absorption of TXP from the spray dilution was significantly reduced when the skin surface was cleaned within 1 hour of exposure. However, if decontamination was delayed to 2 hours post-exposure or longer, then the % of the applied dose taken up and/or permeated was unchanged (at about 20%). This finding is consistent with the results in Figure 2(a) which show that the majority of TXP penetration across the skin had occurred within 4 hours. In contrast, the absorption of TXP from the residue increased progressively with the exposure period; in fact, when the exposure period was 24 hours, the absorption of TXP from the residue was not significantly different to that from the spray dilution.

Conclusions

This study was designed to shed further light on the impact of pesticide properties, exposure time, formulation and skin loading on the potential systemic dose following dermal exposure of re-entry workers to dried residues on crops, plants, fruit, etc. The investigation included parallel measurements using liquid spray dilutions containing the same pesticides, at similar loadings in the same formulations. This comparison is valuable as risk assessments for re-entry workers are typically based on this exposure scenario (i.e., to the liquid spray dilution) rather than on the more realistic contact with a dried residue.

Consistent with previous reports[12], for the four pesticides considered, skin absorption was significantly lower from the dried residue for the majority of scenarios studied. The only exceptions were for CLF when applied at high dermal loading and for TXP applied for 24 hr exposure duration or in a wettable powder (WP) formulation. A key component of the WP used is kaolin powder, a material used in traditional medicine and in numerous skin-care products (e.g., face masks, cosmetics, and in skin barrier formulations). Here, it seems possible that adsorption of the pesticide to the surface of the kaolin particles may become the common, principal factor controlling 'release' of the chemical to the skin, regardless of its application as a spray dilution or as a dried residue.

The kinetics of TXP penetration across the skin reveal some insight into the physics associated with dermal exposure. From the liquid spray dilution, the initial rate of penetration (over, say, the first 4 hours) is more rapid, presumably reflecting a 'metamorphosis' of the formulation as the aqueous phase evaporates, driving the thermodynamic activity of the pesticide higher (and increasing flux). However, once the solvent has gone, TXP penetration slows down as the chemical would now have to undergo a dissolution step before it is able to commence diffusing across the skin. From the residue, on the other hand, TXP is being released under fairly constant conditions as the residue is already dry when the application begins. While there may be some outward movement of water from the

receptor solution towards the skin surface (and this may help with dissolving the pesticide), transepidermal water loss is probably insufficient to completely re-dissolve the TXP in the residue.

Taken together, the results from this research permit three broad conclusions to be drawn. First, with the optimised method employed, transfer of residue to the skin can be achieved reliably and reproducibly, with good efficiency, so that valid comparisons are possible. Second, it is evident that the absorption of pesticide from a dried residue is generally less than when the same chemical is presented to the skin as a spray dilution; this general behaviour, which had been reported previously for one compound only,[12] seems to hold for pesticides differing quite widely in their physicochemical properties and formulation. Despite a large range in the predicted maximum fluxes of the four chemicals across the skin (Table 1), the difference in the absolute quantities penetrated between the most and the least absorbed only ranged from 1.8 to 3.1-fold between spray dilution and residue applications, respectively. Third, the decontamination experiments with TXP reveal that, following exposure to the spray dilution, it is important to clean the skin within 1 hour to significantly reduce potential systemic risk; indeed, removing material from the surface at 30 minutes post-exposure can reduce dermal uptake by 4-fold. With respect to exposure to a dried residue, the data indicate that the sooner decontamination is performed, the greater the reduction in absorption achieved. For example, cleaning the skin after 30 minutes of contact with the residue reduces potential systemic exposure by a factor of 12 compared to that resulting from continual contact for 24 hours. Even if the residue-contaminated skin is only washed at the end of an 8-hour working day, the potential systemic exposure (relative to that at 24 hours) is halved. The cause of the apparent plateau in absorption at around 20% absorbed is unclear; possible explanations may be the formation of crystals on the skin surface following vehicle evaporation or deposition of AI on hair shafts. In fact, both of these phenomena have been observed using light microscopy in further experiments (data not shown).

An additional, noteworthy formulation-related observation concerned the emulsifiable concentrates (EC). The components of these products differ by more than 25% w/v, meaning that EFSA guidance would not allow bridging between the dermal absorption values for these two formulations. However,

the results obtained in this work reveal no differences in skin uptake and absorption from the two formulations for either of the two pesticides considered.

Although the choice of dose (concentration) for a conventional study using aqueous dilutions is relatively easy (as it is dictated by the label recommendations for application rate of the active ingredient and the volume of liquid applied), this is not the case for dried residues, for which the dose would be determined by the level of exposure to the re-entry worker calculated according to the method outlined in the Introduction. As the transfer coefficients for workers differ across different areas of the body depending on the task, and the relevant surface area for these body parts varies widely, the predicted skin loading can also vary. The EFSA guidance on dermal absorption does not provide specific instructions on how to approach this problem but, given that this study suggests that skin loading is not a major determinant of percentage absorption from dry residues, the choice of dose seems less critical. It may therefore be reasonable to apply dried residues at an equivalent dose level to the liquid dilutions in regulatory studies.

Funding Information

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Supporting Information. Tables S1- S6 showing detailed results which correspond to the data presented in figures 3, 4, & 7. Table S7 presents details of analytical and extraction methods.

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Supporting Information

Manuscript: Dermal absorption of pesticide residues

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Table S1: Skin uptake and absorption of CLF from liquid spray dilution application of three formulations (mean ± SD).

CLF formulation - liquid	EC-A	EC-B	WP
Number of replicates (n)	6	6	8
CLF applied (µg)	30	30	30
CLF recovered in swabs (µg)	21.7 ± 2.9	21.8 ± 1.9	24.7 ± 2.4
Total 'dose' recovered (%)	84 ± 11.0	85 ± 5.5	94 ± 7.6
CLF disposition			
SC tape-strips 1-2 (µg)	1.25 ± 0.49	1.28 ± 0.40	1.78 ± 0.53
SC tape-strips 3-15 (µg)	0.54 ± 0.26	0.60 ± 0.24	0.67 ± 0.23
SC tape-strips 1-15 (µg)	1.80 ± 0.69	1.88 ± 0.46	2.45 ± 0.62
Remaining skin (µg)	0.67 ± 0.30	0.75 ± 0.33	0.41 ± 0.31
Receptor phase at 24 hr (µg)	< LOQ[*]	< LOQ	< LOQ
Total CLF uptake/absorption (µg)[#]	1.21 ± 0.43	1.35 ± 0.30	1.08±0.34
% uptake/absorption of CLF applied	4.04 ± 1.42	4.50 ± 1.01	3.60 ± 1.13

[*]< LOQ = below the limit of quantitation of CLF.
[#]Sum of (SC tape-strips 3-15) + (Remaining skin)

Table S2: Skin uptake and absorption of CLF from residue application of three formulations and acetone vehicle. (mean ± SD).

CLF formulation - residue	ECA-A	EC-B	WP	Acetone
Number of replicates (n)	6	4	8	5
CLF applied (µg) *	22.6 ± 1.8	26.2 ± 1.3	35.2 ± 2.0	17.9 ± 3.3
CLF recovered in swabs (µg) *	18.2 ± 2.5	19.7 ± 1.4	32.0 ± 5.6	9.8 ± 2.7
Total 'dose' recovered (%)	92 ± 4.8	87 ± 4.5	98 ± 13	86 ± 3.2
CLF disposition				
SC tape-strips 1-2 (µg) [†]	0.48 ± 0.07	0.58 ± 0.17	1.08 ± 0.57	0.47 ± 0.13
SC tape-strips 3-15 (µg)	0.37 ± 0.14	0.35 ± 0.13	0.42 ± 0.20	0.24 ± 0.03
SC tape-strips 1-15 (µg) [†]	0.85 ± 0.17	0.93 ± 0.19	1.50 ± 0.72	0.71 ± 0.11
Remaining skin (µg)	0.13 ± 0.20	0.20 ± 0.07	0.12 ± 0.08	0.20 ± 0.05
Receptor phase at 24 hr (µg)	<LOQ[@]	<LOQ	<LOQ	<LOQ
Total CLF uptake/absorption (µg)[#]	0.49 ± 0.09	0.55 ± 0.07	0.54 ± 0.15	0.45 ± 0.07
% uptake/absorption of CLF applied *	2.17 ± 0.31	2.09 ± 0.29	1.54 ± 0.44	2.55 ± 0.54

[*]Significantly different ($p < 0.01$) between formulation type (1-way ANOVA).
[†]Significantly different ($p < 0.05$) between formulation type (1-way ANOVA).
[@]< LOQ = below the limit of quantitation of CLF.
[#]Sum of (SC tape-strips 3-15) + (Remaining skin)

Table S3: Skin uptake and absorption of TXP from liquid spray dilution application of three formulations and from an aqueous solution (mean ± SD).

TXP formulation - liquid		EC-A	EC-B	WP	Aq. solution
Number of replicates		5	4	5	4
TXP applied (µg)		30	30	30	30
TXP recovered in swabs (µg) *		18.8 ± 1.8	17.1 ± 1.95	20.3 ± 0.8	13.1 ± 0.8
Total 'dose' recovered (%)		87 ± 5.4	80 ± 4.1	84 ± 4.1	65 ± 5.2
TXP disposition					
SC tape-strips 1-2 (µg)		1.10 ± 0.33	0.82 ± 0.12	0.97 ± 0.13	1.12 ± 0.47
SC tape-strips 3-15 (µg)		0.55 ± 0.38	0.56 ± 0.24	0.43 ± 0.20	0.36 ± 0.07
SC tape-strips 1-15 (µg)		1.65 ± 0.70	1.39 ± 0.28	1.40 ± 0.29	1.48 ± 0.53
Remaining skin (µg)		1.09 ± 0.29	1.18 ± 0.41	0.87 ± 0.25	1.18 ± 0.38
Receptor phase (µg)	2 hr†	1.07 ± 0.35	0.66 ± 0.46	0.50 ± 0.13	0.80 ± 0.39
	3 hr*	1.72 ± 0.52	1.38 ± 0.09	0.76 ± 0.15	1.25 ± 0.64
	4 hr*	2.14 ± 0.68	1.73 ± 0.18	0.98 ± 0.20	1.57 ± 0.81
	5 hr†	2.52 ± 0.88	1.91 ± 0.21	1.16 ± 0.25	1.75 ± 0.93
	6 hr†	2.78 ± 0.90	2.20 ± 0.23	1.27 ± 0.25	2.00 ± 1.04
	7 hr†	2.93 ± 0.92	2.24 ± 0.25	1.27 ± 0.20	2.14 ± 1.07
	8 hr†	3.09 ± 1.02	2.51 ± 0.26	1.35 ± 0.25	2.26 ± 1.12
	24 hr*	4.04 ± 1.09	3.63 ± 0.23	1.90 ± 0.42	2.94 ± 1.29
Total TXP uptake/absorption # (µg) *		5.69 ± 0.90	5.38 ± 0.79	3.20 ± 0.78	4.49 ± 1.16
% uptake/absorption of TXP applied *		19.0 ± 3.0	17.9 ± 2.6	10.7 ± 2.6	15.0 ± 3.9

*Significantly different ($p < 0.01$) between formulation type (1-way ANOVA).
†Significantly different ($p < 0.05$) between formulation type (1-way ANOVA).
#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

Table S4: Skin uptake and absorption of TXP from residue application of three formulations and from aqueous solution (mean ± SD).

TXP formulation - residue		EC-A	EC-B	WP	Aq. solution
Number of replicates		5	7	9	3
TXP applied (µg)		27.1 ± 1.5	29.1 ± 3.1	27.2 ± 5.7	11.3 ± 1.0
TXP recovered in swabs (µg)		18.9 ± 3.5	14.3 ± 2.1	15.7 ± 3.4	3.3 ± 1.0
Total 'dose' recovered (%)		90 ± 12	76 ± 3.1	82 ± 4.4	83 ± 3.5
TXP disposition					
SC tape-strips 1-2 (µg)		0.66 ± 0.24	0.85 ± 0.32	1.16 ± 0.84	0.19 ± 0.10
SC tape-strips 3-15 (µg)		0.29 ± 0.14	0.41 ± 0.14	0.37 ± 0.20	0.04 ± 0.01
SC tape-strips 1-15 (µg)		0.95 ± 0.38	1.26 ± 0.42	1.54 ± 0.93	0.23 ± 0.09
Remaining skin (µg)		0.76 ± 0.40	0.75 ± 0.60	0.61 ± 0.29	0.06 ± 0.11
Receptor Phase (µg)	2 hr	0.72 ± 0.89	0.69 ± 0.40	0.71 ± 0.46	< LOQ[@]
	3 hr	0.99 ± 1.06	1.06 ± 0.39	0.95 ± 0.55	< LOQ
	4 hr	1.23 ± 1.02	1.26 ± 0.43	1.07 ± 0.61	< LOQ
	5 hr	1.35 ± 1.09	1.46 ± 0.46	1.20 ± 0.62	< LOQ
	6 hr	1.49 ± 1.26	1.68 ± 0.56	1.31 ± 0.68	< LOQ
	7 hr	1.56 ± 1.24	1.83 ± 0.55	1.36 ± 0.68	0.06 ± 0.11
	8 hr	1.64 ± 1.26	2.00 ± 0.58	1.42 ± 0.72	0.09 ± 0.15
	24 hr	2.27 ± 1.34	2.76 ± 0.66	1.86 ± 0.88	0.25 ± 0.25
Total TXP uptake/absorption (µg)[#]		3.32 ± 1.33	3.92 ± 0.99	2.84 ± 1.00	0.35 ± 0.26
% uptake/absorption of TXP applied		12.2 ± 4.5	13.4 ± 2.8	10.3 ± 2.7	3.0 ± 2.3

'Aq. solution' was not included in statistical analysis

[†]Significantly different ($p < 0.05$) between formulation type (1-way ANOVA).

[#]Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

[@]< LOQ = below the limit of quantitation of TXP.

Table S5: Results from the skin surface decontamination experiments performed with TXP liquid spray dilution (mean ± SD)

TXP Liquid							
Number of replicates		3	3	7	7	3	3
Decontamination Time		0.5	1	2	4	8	24
TXP recovered in swabs (µg)		20.6 ± 1.28	22.9 ± 4.03	19.3 ± 2.03	20.1 ± 9.32	18.1 ± 3.47	18.8 ± 1.17
TXP disposition							
SC tape-strips 1-2 (µg)		0.65 ± 0.21	0.42 ± 0.12	0.63 ± 0.30	0.66 ± 0.18	0.71 ± 0.57	0.39 ± 0.27
SC tape-strips 3-15 (µg)		0.10 ± 0.02	0.19 ± 0.12	0.22 ± 0.14	0.29 ± 0.11	0.18 ± 0.17	0.24 ± 0.04
SC tape-strips 1-15 (µg)		0.75 ± 0.23	0.61 ± 0.24	0.85 ± 0.35	0.94 ± 0.18	0.89 ± 0.74	0.63 ± 0.31
Remaining skin (µg)		0.62 ± 0.17	0.77 ± 0.36	1.44 ± 0.65	1.10 ± 0.48	1.32 ± 0.78	0.72 ± 0.19
Receptor Phase (µg)	2 hr	0.63 ± 0.04	1.66 ± 0.46	2.00 ± 1.09	2.10 ± 0.99	0.79 ± 0.80	1.84 ± 0.18
	3 hr	0.82 ± 0.15	1.98 ± 0.49	2.85 ± 1.45	2.82 ± 1.24	1.18 ± 1.23	2.31 ± 0.19
	4 hr	0.90 ± 0.19	2.11 ± 0.53	3.30 ± 1.56	3.34 ± 1.52	1.57 ± 1.47	2.67 ± 0.10
	5 hr	0.84 ± 0.10	2.17 ± 0.55	3.46 ± 1.63	3.67 ± 1.66	1.84 ± 1.65	2.96 ± 0.08
	6 hr	0.89 ± 0.10	2.30 ± 0.57	3.70 ± 1.76	4.01 ± 1.86	2.16 ± 1.80	3.24 ± 0.08
	7 hr	0.91 ± 0.16	2.30 ± 0.57	3.85 ± 1.84	4.18 ± 1.99	2.35 ± 2.06	3.35 ± 0.02
	8 hr	0.86 ± 0.12	2.29 ± 0.63	3.89 ± 1.89	4.19 ± 2.01	2.52 ± 2.12	3.51 ± 0.10
	24 hr	1.10 ± 0.20	2.25 ± 0.63	4.47 ± 2.37	4.65 ± 2.36	4.02 ± 0.36	5.30 ± 0.17
Total TXP uptake/absorption (µg)#		1.81 ± 0.05	3.22 ± 0.40	6.13 ± 2.78	6.03 ± 2.79	5.52 ± 3.00	6.27 ± 0.05
% uptake/absorption of TXP applied		6.04 ± 0.17	10.7 ± 1.33	20.4 ± 9.26	20.1 ± 9.32	18.4 ± 9 .99	20.9 ± 0.17

#Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

Table S6: Results from the skin surface decontamination experiments performed with TXP residue (mean ± SD).

TXP Residue							
Number of replicates		3	3	7	6	6	3
Decontamination Time		0.5	1	2	4	8	24
TXP applied (µg)		28.8 ± 0.59	26.8 ± 2.64	28.3± 2.48	27.9 ± 3.04	27.6 ± 1.45	31.4 ± 1.40
TXP recovered in swabs (µg)		18.3 ± 1.16	17.7 ± 1.98	18.2 ± 0.87	15.9 ± 2.10	17.5 ± 3.69	14.8 ± 1.61
TXP disposition							
SC tape-strips 1-2 (µg)		0.23 ± 0.26	0.37 ± 0.11	0.56 ± 0.37	0.57 ± 0.42	0.63 ± 0.21	0.23 ± 0.05
SC tape-strips 3-15 (µg)		0.02 ± 0.03	0.16 ± 0.12	0.16 ± 0.10	0.13 ± 0.12	0.24 ± 0.13	0.22 ± 0.15
SC tape-strips 1-15 (µg)		0.25 ± 0.24	0.53 ± 0.20	0.72 ± 0.45	0.70 ± 0.54	0.87 ± 0.33	0.45 ± 0.20
Remaining skin (µg)		0.20 ± 0.17	0.13 ± 0.05	0.36 ± 0.32	0.47 ± 0.35	0.51 ± 0.23	0.50 ± 0.02
Receptor Phase (µg)	2 hr	0.06 ± 0.10	1.03 ± 0.21	0.80 ± 0.61	1.01 ± 1.12	0.81 ± 0.84	1.63 ± 0.19
	3 hr	0.09 ± 0.16	1.12 ± 0.22	1.11 ± 0.70	1.30 ± 1.35	1.07 ± 1.00	1.89 ± 0.24
	4 hr	0.09 ± 0.15	1.21 ± 0.23	1.22 ± 0.73	1.58 ± 1.37	1.30 ± 0.97	2.15 ± 0.22
	5 hr	0.09 ± 0.16	1.24 ± 0.22	1.29 ± 0.73	1.77 ± 1.52	1.43 ± 1.03	2.42 ± 0.33
	6 hr	0.16 ± 0.15	1.29 ± 0.30	1.36 ± 0.77	1.93 ± 1.93	1.58 ± 1.17	2.76 ± 0.42
	7 hr	0.15 ± 0.14	1.26 ± 0.27	1.36 ± 0.79	1.95 ± 1.56	1.65 ± 1.17	2.83 ± 0.47
	8 hr	0.18 ± 0.16	1.25 ± 0.29	1.37 ± 0.69	2.04 ± 1.65	1.79 ± 1.19	2.99 ± 0.43
	24 hr	0.22 ± 0.20	1.30 ± 0.35	1.36 ± 0.62	2.04 ± 1.87	2.43 ± 1.26	5.28 ± 0.81
Total TXP uptake/absorption (µg)[#]		0.44 ± 0.24	1.58 ± 0.40	1.88 ± 0.85	2.63 ± 1.89	3.18 ± 1.18	6.00 ± 0.67
% uptake/absorption of TXP applied		1.53 ± 0.85	5.85 ± 1.06	6.60 ± 2.68	9.41 ± 6.94	11.5 ± 4.04	19.1 ± 1.46

[#]Sum of (SC tape-strips 3-15) + (Remaining skin) + (Receptor phase at 24 hr).

Table S7: A Shimadzu LC-20101A HPLC was used (with a 25 cm C18 column HiQ sil C18HS, having particle and pore sizes of 5 µm and 100 Å, respectively) for all pesticide analyses in this study. The relevant conditions for the assays are provided in the table below. The injection volume was 50 µL for all samples.

Compound	Mobile phase	Oven temp. (°C)	UV detection wavelength (nm)	Flow rate (mL/min)	Retention time (min)	Extraction solution[#]
CLF	70/30 ACN:H2O	25	226	1.0	8.6	70/30 ACN:H2O
CLF[*]	75/25 ACN:H2O	25	226	1.0	6.7	
DFZ	70/30 ACN:H2O	35	212	1.5	6.9	80/20 ACN:H2O
PPZ	70/30 ACN:H2O	25	220	1.5	6.1	70/30 ACN:H2O
TXP	60/40 ACN:0.1%H_2PO_4	25	280	1.0	8.5	60/40 ACN:H2O

[*]*Method used specifically for CLF analysis of extracted stratum corneum tape strips.*

[#]*Tape strip and remaining skin samples were added to 1 or 4mL, respectively, of the relevant extraction solution and left on a shaker overnight. Samples were then filtered before quantification of AI by HPLC.*

Table S8: Constituents of the concentrate formulations tested in this study.

Type	Name	CAS number
EC-A		
Emulsifier	Castor oil, ethoxylated	61791-12-6
Emulsifier	Calcium dodecylbenzene sulphonate	26264-06-2
Emulsifier	Tristyrylphenol ethoxylated	99734-09-5
Solvent	1-Phenylethan-1-one	98-86-2
Solvent	Solvent naphtha (petroleum), heavy aromatic	64742-94-5
EC-B		
Emulsifier	Castor oil, ethoxylated	61791-12-6
Emulsifier	Calcium dodecylbenzene sulphonate	26264-06-2
Emulsifier	Tristyrylphenol ethoxylated	99734-09-5
Solvent	1-Phenylethan-1-one	98-86-2
Adjuvant	Oleic acid methyl ester	112-62-9
WP		
Dispersant	Lignin, sodium sulphate	9009-75-0
Wetting agent	Butylnaphthalenesulphonic acid salt	25638-17-9
Filler	Powdered kaolin	1332-58-7